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단 신

플루오르를 가진 삼중고리 Enediyne 화합물에 대한 화학적 반응성과 생물학적 활성

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Chemical Reactivity and Biological Activities for Tricyclic Enediyne Compound Possessing Fluorine

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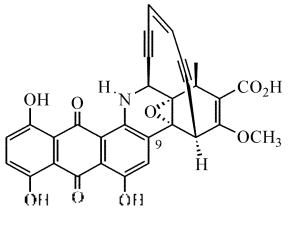
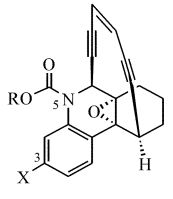
Dynemicin A (**1**), a new enediyne-containing compound isolated from the fermentation broth of *micromonospora chersina* is a potent antitumor antibiotic with unique molecular structure and fascinating mode of action.[1] The activation of **1** is triggered by epoxide opening induced by bioreduction of anthraquinone, followed by developing electron density at C9.[2] Consequently, the constrained 10-membered enediyne generates a benzenoid diradical via Bergman cycloaromatization reaction[3], and the radical initiates DNA cleavage. Electron density at C9 is dependent upon electron releasing power of both nitrogen and oxygen on benzene ring. We reported previously the substituent effect for activation of tricyclic enediyne compound with fluorine under weak acidic condition.[4] Compound **2** with fluorine at C3 showed significantly a fast activation in comparison with unsubstituted compound **3**[2b]. Here, we note chemical reactivity, and biological activities for a new enediyne compound **5**.



Dynemicin A (1)

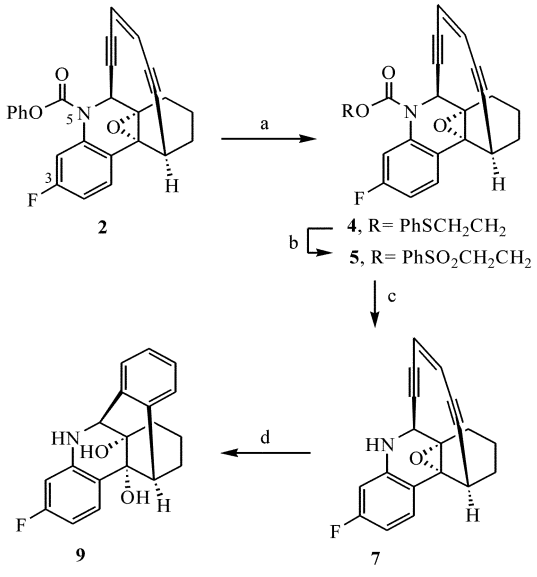


2. R = Ph, X = F
3. R = Ph, X = H
5. R = $PhSO_2CH_2CH_2$, X = F
6. R = $PhSO_2CH_2CH_2$, X = H

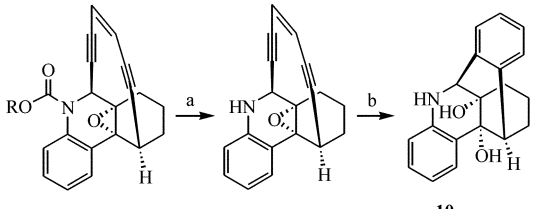
Fig. 1. Dynemicin A and its tricyclic models.

Synthesis, *N*-deprotection and Chemical Reactivity of the New Enediyne 5. The introduction of base-labile group at *N*5 was performed by a known method[2b] (*Scheme* 1). Compound **2**[4] was treated with sodium hydride and 2-(phenylthio)ethanol to give sulfide **4** in quantitative yield. Continuously, oxidation of the sulfide with *m*-chloroperoxybenzoic acid (*m*CPBA) produced the enediyne **5**.

This enediyne was treated with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in wet benzene and 1,4-cyclohexadiene expecting the formation of cycloaromatized compound **9** via *N*-deprotected free amine **7**. Surprisingly, **7** was clearly isolated instead of **9**. Furthermore, unsubstituted amine **8** was also isolated from enediyne **6**[2b] under basic condition (*Scheme* 2). The compounds **7** and **8** are the first identified free amines among enediyne models



Scheme 1. (a) $PhSCH_2CH_2OH$ (2.0 equiv.), NaH (2.0 equiv.), THF, 25 °C; (b) *m*CPBA (2.5 equiv.), CH_2Cl_2/sat. $NaHCO_3$ (1:1), 0 °C; (c) DBU (2.0 equiv), benzene, 20 °C; (d) SiO_2, wet benzene/1,4-cyclohexadiene (3/1), 20 °C.



Scheme 2. (a) DBU (2.0 equiv), benzene, 20 °C; (b) 1% HCl, benzene/1,4-cyclohexadiene (3/1), 20 °C.

related to dynemicin A so far as we know. Until now, it has been known that they are very unstable and cannot be isolated.[5] Presumably, basic condition deactivates the epoxide opening and then, stabilizes the compounds. Expectedly, Bergman cyclization for **7** gave diol **9** under weak acidic condition within 1 hour. On the other hand, the unsubstitued compound **8** did not give any product in a long time under the same condition. But, when 1% HCl was added to the solution, the free amine **8** spot disappeared within 5 min, converted to a new one which corresponded putatively to diol **10** on TLC. Unfortunately, compound **10** was not identified by spectroscopic method. But, the result for free amine

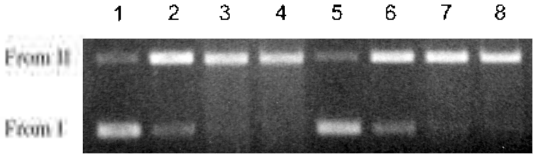


Fig. 2. DNA interaction with enediynes **5** and **6**. pUC18 DNA was incubated for 48 h at 37 °C with compounds **5** and **6** in buffer (50 mM Tris-HCl, pH 8.5) and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain): lane 1 and 5, DNA control; lane 2, **6** [0.1 mM]; lane 3, **6** [1 mM]; lane 4, **6** [10.0 mM]; lane 6, **5** [0.1 mM]; lane 7, **5** [1.0 mM]; lane 8, **5** [10.0 mM]. Form II, open-circular DNA; Form I, supercoiled DNA.

reactivity suggested that fluorine should activate the epoxide opening and accelerate cycloaromatization.

DNA Cleavage and Cytotoxicity Studies. The DNA cleaving property for the enediyne **5** was examined and compared with the unsubstituted **6**. The pUC18 supercoiled DNAs with enediynes **5**, **6** were incubated at 37 °C under basic conditions. After 48 h, significant DNA cleavages were resulted in the formation of form II DNA (*Fig.* 2). But, any activity difference was not detected between **5** and **6**.

The cytotoxicities of enediynes **5** and **6** against several human tumor cell lines were determined using adriamicin as a control drug (*Table* 1). Our new compound **5** showed lower potency than adriamicin. Moerover, any striking difference in potencies was not observed between **5** and **6**.

In conclusion, even though compound **5** with fluorine represented relatively better reactivity toward cycloaromatization in comparison with **6**, the two showed similar activities toward *in vitro* biological tests. It has been assumed that biological factors (e.g., drug-DNA intercalation) overwhelmed chemical reactivity for the two compounds.

EXPERIMENTAL SECTION

Genenral Techniques. NMR spectra were recorded on a Bruker DPX-300 or 500 instrument. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) under UV light. All new compounds were identified by spectroscopic methods.

Table 1. Cytotoxicities of Enediynes **5**, **6**, and Adriamicin

Cell type	Cell line	ED_{50} (μM)		
		5	**6**	adriamicin
Non-small cell lung	A-549	0.16	0.14	0.01
Ovrian	SKOV-3	0.62	0.58	0.14
Melanoma	SKMEL-2	2.00	0.74	0.03
CNS	XF-498	0.24	0.25	0.09
Colon	HCT-15	0.20	0.35	0.20

Synthesis of compound 4. To a suspension of NaH (38.9 mg of 60% dispersion in mineral oil. 0.97 mmol) in dry THF (2 mL) was added 2-(phenylthio)ethanol (0.13 mL, 0.97 mmol) followed by stirring at 25 °C for 5 min. The resulting solution was added to a solution of **2** (200 mg, 0.49 mmol) in dry THF (4 mL). After stirring at 25 °C for 10 min, the reaction mixture was diluted with ethyl ether (20 mL), poured into H_2O (50 mL), and extracted with ethyl ether (2×20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 25% ethyl ether in hexane) to give the product **4** in quantitative yield. R_f = 0.45 (silica gel, 25% ethyl ether in hexane); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.68 (dd, J = 8.8, 6.2 Hz, 1H, aromatic), 7.38-7.23 (m, 3H, aromatic), 7.21-7.09 (m, 2H, aromatic), 7.17 (td, J = 8.6, 2.7 Hz, 1H, aromatic), 6.74-6.69 (m, 1H, aromatic), 5.98 (dd, J = 9.9, 1.4 Hz, 1H, olefinic), 5.86 (dd, J = 9.9, 1.4 Hz, 1H, olefinic), 5.35 (br s, 1H, *CH*N), 4.31-4.25 (m, 1H, CH_2O), 4.28-4.15 (m, 1H, CH_2O), 3.97 (br s, 1H, CH_2CH), 3.33-3.23 (m, 2H, SCH_2), 2.26-2.19 (m, 1H, CH_2), 2.07-1.98 (m, 1H, CH_2), 1.79-1.63 (m, 3H, CH_2), 1.54-1.45 (m, 1H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.2 ($^1J_{CF}$=244 Hz), 153.2, 135.0, 129.3, 129.2, 128.5, 126.1, 125.8, 122.3, 119.5, 118.7, 112.5 ($^2J_{CF}$=21 Hz), 111.8 ($^2J_{CF}$=21 Hz), 102.3, 93.7, 91.0, 88.7, 69.6, 67.9, 64.6, 60.1, 48.7, 28.6, 22.8, 22.2, 15.2.

Synthesis of compound 5. To a solution of **4** (186 mg, 0.40 mmol) in dichloromethane (2.6 mL) and saturated aqueous sodium bicarbonate (2.6 mL) was added *m*CPBA (70%, 243 mg, 0.99 mmol) followed by stirring at 0 °C for 10 min. The reaction mixture was poured into saturated aqueous sodium bicarbonate (15 mL) and extracted with dichloromethane (2×15 mL). The combined organic layers were dryed (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 75% ethyl ether in petroleum ether) to provide **5** (119 mg, 60%). R_f = 0.38 (silica gel, 75% ethyl ether in hexane); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (d, J=7.6 Hz, 2H, aromatic), 7.75-7.69 (m, 2H, aromatic), 7.63 (t, J=7.6 Hz, 2H, aromatic), 7.17 (dd, J=10.6, 2.7 Hz, 1H, aromatic), 7.06 (td, J=8.5, 2.7 Hz, 1H, aromatic), 6.01 (dd, J= 9.9, 1.7 Hz, 1H, olefinic), 5.90 (dd, J=9.9, 1.7 Hz, 1H, olefinic), 5.20 (br s, 1H, *CH*N), 4.43 (m, 1H, CH_2O), 4.34 (m, 1H, CH_2O), 4.00 (br s, 1H, CH_2CH), 3.82-3.78 (m, 2H, SO_2CH_2), 2.25-2.20 (m, 1H, CH_2), 2.10-2.05 (m, 1H, CH_2), 1.82-1.78 (m, 1H, CH_2), 1.75-1.69 (m, 2H, CH_2), 1.55-1.51 (m, 1H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.2 ($^1J_{CF}$ = 244 Hz), 152.6, 139.0, 134.1, 129.5, 129.2, 128.3, 127.5, 125.8, 124.5, 122.3, 112.6 ($^2J_{CF}$ = 25 Hz), 111.8 ($^2J_{CF}$=25 Hz), 102.3, 93.5, 91.0, 88.7, 69.5, 60.6, 59.9, 53.8, 48.7, 28.6, 22.6, 22.1, 15.2.

Synthesis of free amine 7. To a solution of enediyne **5** (20 mg, 0.04 mmol) in benzene (1.5 mL) was added DBU (12 mL, 0.08 mmol) at 20 °C. The reaction progress was monitored at a proper interval by TLC. When the reaction was completed the solution was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 75% ethyl ether in hexane containing 1% triethylamine) to give the amine **7** (7.8 mg, 69%). R_f = 0.75 (silica gel, 75% ethyl ether in hexane); ^{1}H NMR (300MHz, DMSO-d_6): δ 7.49 (dd, J=8.6, 6.4 Hz, 1H, aromatic), 6.61 (d, J=2.9 Hz, 1H, N*H*), 6.44 (td, J=8.6, 2.6 Hz, 1H, aromatic), 6.35 (dd, J=11.0, 2.6 Hz, 1H, aromatic), 5.97 (s, 2H, olefinic), 4.29

(br s, 1H, NC*H*), 3.90 (br s, 1H, CH_2CH), 2.14 (dd, *J*=16.1, 7.1, 1H, CH_2), 2.01-1.88 (m, 1H, CH_2), 1.78-1.55 (m, 3H, CH_2), 1.51-1.40 (m, 1H, CH_2); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 162.5 ($^1J_{CF}$=240 Hz), 145.2, 139.5, 131.5, 124.2, 123.2, 103.7 ($^2J_{CF}$=22 Hz), 103.1, 101.2 ($^2J_{CF}$=25 Hz), 98.1, 90.3, 86.7, 69.5, 60.0, 47.4, 28.4, 23.6, 23.4, 15.3.

Synthesis of free amine 8. Compound **8** was prepared from enediyne **6** in 54% yield in a same manner as described for **7**. R_f= 0.75 (silica gel, 75% ethyl ether in hexane); ^{1}H NMR (300MHz, DMSO-d_6): δ 7.43 (br d, *J*=7.7 Hz, 1H, aromatic), 6.99 (td, *J*=8.5, 1.3 Hz, 1H, aromatic), 6.61 (td, *J*=8.5, 1.1 Hz, 1H, aromatic), 6.53 (br d, *J*=8.0 Hz, 1H, aromatic), 6.22 (d, *J*=2.8 Hz, 1H, N*H*), 5.89 (s, 2H, olefinic), 4.19 (br s, 1H, NC*H*), 3.86 (br s, 1H, CH_2CH), 2.14 (dd, *J*=15.4, 6.9, 1H, CH_2), 2.00-1.89 (m, 1H, CH_2), 1.80-1.61 (m, 3H, CH_2); 1.47-1.40 (m, 1H, CH_2); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 143.2, 128.3, 126.8, 124.0, 123.1, 121.1, 117.1, 114.9, 103.2, 98.6, 90.2, 86.6, 69.8, 60.3, 47.6, 28.2, 23.7, 23.4, 15.3.

Cycloaromatization of the intermediate 7. To a solution of amine **7** (7.8 mg, 0.027 mmol) in wet benzene (1.5 mL) and 1,4-cyclohexadiene (0.5 mL) was added a catalytic amount of silica gel at 20 °C. After 40 min, the reaction was completed and the solution was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 67% ethyl ether in hexane) to give the diol **9** (4.0 mg, 48%). R_f= 0.35 (silica, 67% ethyl ether in hexane); ^{1}H NMR (500MHz, $CDCl_3$): δ 7.92 (d, *J*=7.5 Hz, 1H, aromatic), 7.60-7.54 (m, 2H, aromatic), 7.19-7.14 (m, 1H, aromatic), 6.92 (d, *J*=7.3 Hz, 1H, aromatic), 6.61 (td, *J*=8.5, 2.4 Hz, 1H, aromatic), 6.51 (td, *J*=8.5, 2.4 Hz, 1H, aromatic), 4.32 (br s, 1H, NC*H*), 4.28-4.15 (br, 1H, N*H*), 4.16 (s, 1H, O*H*), 3.55 (br s, 1H, CH_2CH), 2.75 (s, 1H, O*H*), 2.49 (td, *J*=13.6, 6.3 Hz, 1H, CH_2), 2.32 (dt, *J*=13.6, 3.6 Hz, 1H, CH_2), 2.25 (td, *J*=14.1, 6.3 Hz, 1H, CH_2), 1.84 (dd, *J*=13.8, 5.2 Hz, 1H, CH_2), 1.70 (dd, *J*=13.8, 5.2 Hz, 1H, CH_2), 1.47-1.44 (m, 1H, CH_2; ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 163.3 ($^1J_{CF}$=244 Hz), 139.1, 134.3, 130.0, 129.4, 128.9, 127.6, 127.5, 127.4, 126.2, 108.1 ($^2J_{CF}$=22 Hz), 102.2 ($^2J_{CF}$=25 Hz), 73.1, 63.2, 52.8, 32.6, 30.4, 27.8, 19.0.

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